Discovery of novel pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors based on a pharmacokinetic property-driven optimization

Hui Xie[a] *, Saogao Zeng*[a]*, Lili Zeng*[a]*, Xiaobing Lan*[a]*, Guicheng Zhang*[a]*, , Li Liu*[a]*, , Xin Lu*[a]*, Na Chen*[a]*, Zhiyuan Li*[a]*, Zengzhao Tu*[a]*, Hongjiang Xu*[b]*, Ling Yang*[b] *, Xiquan Zhang*[b]*, Wenhui Hu*[a,]***

[a]Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, 190 Kai Yuan Avenue, Guangzhou Science Park, Guangzhou, 510530, China

[b]Jiangsu Chia-Tai Tianqing Pharmaceutical Co. Ltd, No. 8 Julong North Rd. Xinpu Lianyungang Jiangsu**,** 222006, China.

Email address: xie_hui@gibh.ac.cn

RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

* To whom correspondence should be addressed. Phone: +86-20-32015211. Fax: +86-20-32015299. Email address: hu_wenhui@gibh.ac.cn.

Followed pharmacological evaluation exposed an extensive hepatic first pass effect within our recently disclosed DPP-IV inhibitors bearing thienopyrimidine scaffold. Through scaffold replacement with pyrrolopyrimidine, compound **1a** had substantially improved the metabolic stability (from 6.6% to 65.07%), yet with severely poor absorptive property. Further modification by incorporation with varied

substituents and structure conversion yielded both permeable and metabolic stable compounds. The whole pharmacokinetic- property based optimization had succeeded in balancing overall properties and resulted in the compound **1j**, that with excellent efficacy to be a potential anti-diabetic candidate.

Introduction

With more than 220 million people affected, diabetes has emerged as an epidemic of this century and becomes a huge health problem worldwide. Type 2 diabetes (T2D, formerly called non-insulin-dependent or adult-onset) results from the body's ineffective use of insulin and comprises 90% of people who have diabetes. The growing number of deaths attributable to diabetes reflects the insufficient glycemic control achieved by the past and current treatments [1]. Thus new therapeutic agents are needed to develop to effectively combat diabetes. Glucagons-like peptide-1 (GLP-1) [2] is an incretin secreted from the L cells of the small intestine in a glucose-dependent manner. This hormone can exert several biological functions including stimulation of insulin secretion, inhibition of glucagons secretion, induction of satiety, retardation of gastric emptying and stimulating the regeneration and differentiation of islet β cell [3]. However, with such attracting anti-diabetic functions, GLP-1 is quickly degraded through dipeptidyl peptidase IV (DPP-IV) by being cleaved a dipeptide from the N-terminus and with a half live of 0.5 min under normal physiological condition [4].

DPP-IV inhibitors are new oral glucose-lowing agents acting by reducing the turnover and prolonging the life time of GLP-1. With good patient compliance and less risk of hypoglycemia or other side effects, DPP-IV inhibitors have been demonstrated to be an effective and safe way of blood glucose control [5]. To date, Sitagliptin 1 [6], Vildaglipin 2 [7], Saxagliptin 3 [8], Alogliptin 4 [10] and Linagliptin 5 [9] are already on the market in many countries (Figure 1).

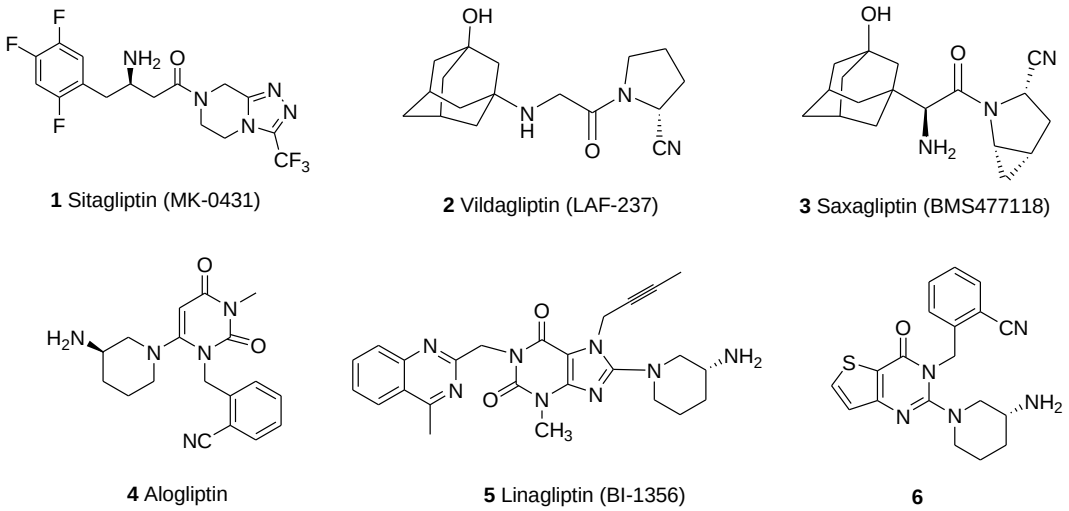


Figure 1. Representative DPP-IV inhibitors.

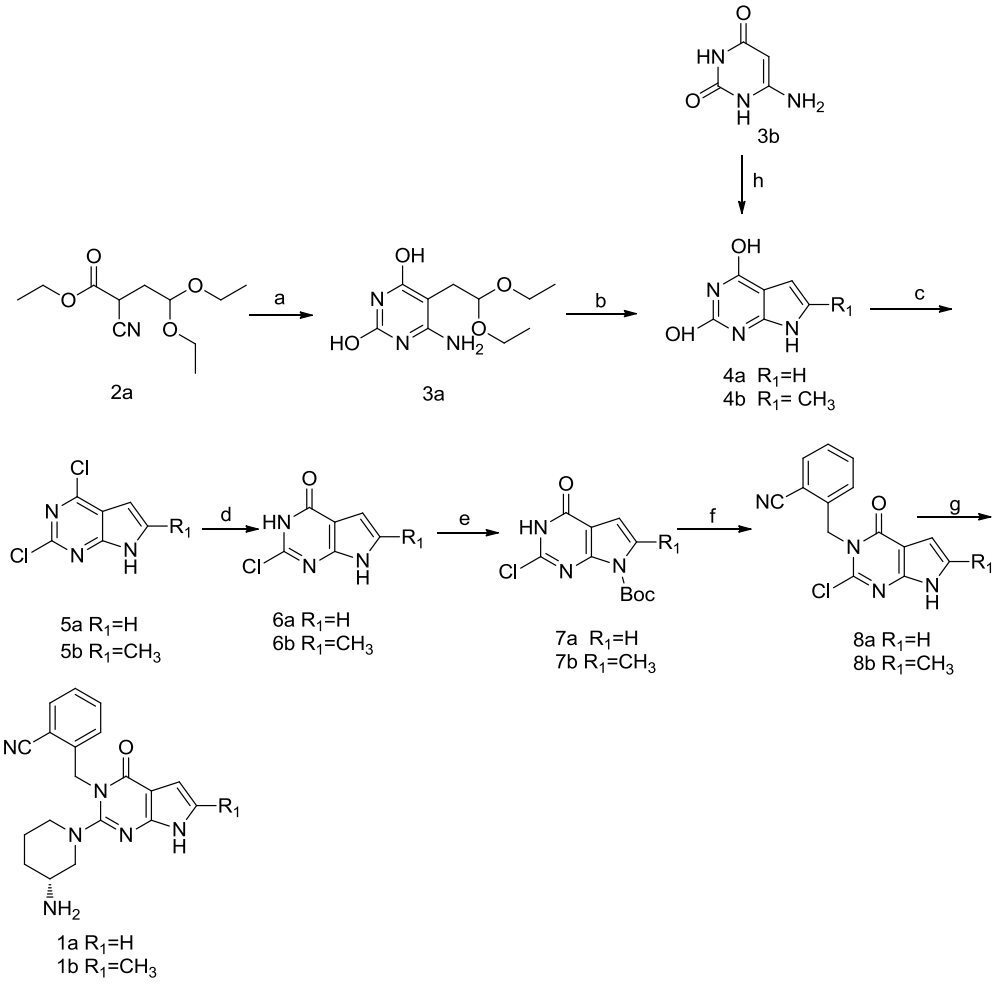
To combat a chronic disease, a drug is always preferred to have similar beneficial effects but with less toxicity at low doses. Even though there are several drugs on the market, highly potent and selective DPP-IV inhibitors are still in great need. Therefore many novel DPP-IV inhibitors have been synthesized based on the structures of 1, 2 and 3 in recent years [11], which in turn leaves a large amount of chemical space to explore as few analogues of Alogliptin have been disclosed. In a previous report from our laboratory [12], we discovered a novel series of compounds bearing thienopyrimidine scaffold as DPP-IV inhibitors which were intrinsically potent against DPP-IV and selective over the other dipeptidyl peptidase (DPP) family of enzymes (see compound **6** in Figure 1 as an example). However, in the followed pharmacological evaluations, compound **6** was found to just have a 23.3% bioavailability in rat [12]. Since DPP-IV inhibitors are oral anti-diabetic therapeutic agents, this fact raised our concerns about putative low in vivo efficacy. In fact, we suspected the insufficient oral bioavailability might be the reason that highly potent compound **6** (IC50=0.33 nM) [12] just exerted similar in vivo efficacy as Alogliptin. In our continued medicinal chemistry effort to develop anti-diabetic candidates, we conducted a pharmacokinetic (PK) property-driven optimization on this scaffold and led to a new scaffold of pyrrolopyrimidine analogues. By replacing the thienopyrimidine scaffold with its isostere pyrrolopyrimidine, we successfully solved the severe metabolic biotransformation problem existed in thienopyrimidine analogues. We further employed several different substituents on the new scaffold to

increase their permeability and to reduce the transporter-mediated efflux in order to avoid from low oral bioavailability caused by poor absorption. This PK-driven optimization had led to a new series of highly potent and selective DPP-IV inhibitors as represented by compound **1j** which has a good balance of activity, selectivity, PK, and in vivo efficacy. Herein, we wish to report the synthesis and the process of this PK-driven optimization on these new pyrrolopyrimidine analogues: their pharmacological activities, PK, and pharmacodynamic (PD) evaluations.

Chemistry

The synthesis of compounds **1a** and **1b** are outlined in **Scheme 1**. Briefly, The synthesis of **1a** began with commercially available ethyl 2-cyano-4,4-diethoxybutanoate **2a**, which was heated with urea at 80 °C , then acidified with conc. HCl to give the diol compound **4a**. The synthesis of **1b** began with commercially available 6-aminopyrimidine-2, 4(1H, 3H)-dione **3b**, which was heated with 1-chloro-2-Propanone to get compound **4b**. Chlorination of **4a** and **4b** with phosphoryl trichloride yielded **5a** and **5b**, which were hydrolyzed with aqueous sodium hydroxide to give key intermediates **6a** and **6b**. Selective *N*-alkylation was performed using a previously published method [13] to produce compounds **8a** and **8b**. The final compounds, **1a** and **1b** were obtained in high yields by the amination of the chloro precursors **8a** and **8b** with 3-(*R*)-aminopiperidine.

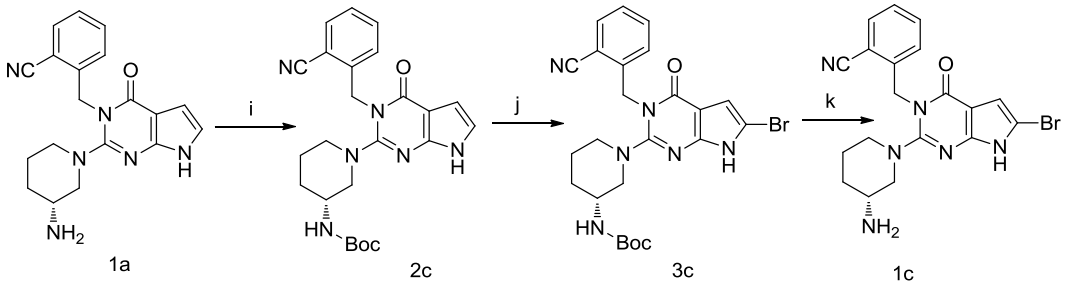
Scheme 1. Synthesis of compounds **1a** and **1b.**



Reagents: (a) EtOH, Na, urea, 80°C; (b) conc. HCl; (c) 1N KOH; (d) $POCl_3$, DIEA, toluene; (e) $(Boc)_2O$, DMAP, Et_3N; (f) 2-CNPhCH_2Br, NaH, LiBr; (g) 3-(*R*)-aminopiperidine, $NaHCO_3$, 150°C; (h) 1-chloro-2-Propanone, NaOAc.

The synthesis of compounds **1c** is outlined in **Scheme 2**. The synthesis of **1c** began with the compounds **1a**, which was treated with $(Boc)_2O$, then bromized with NBS to get the final compound **1c**.

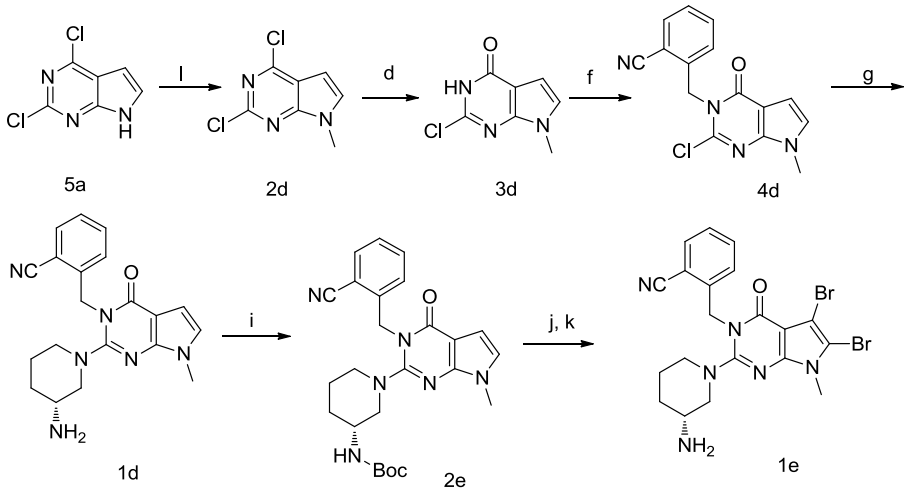
Scheme 2. Synthesis of compound **1c.**



Reagents: (i) $(Boc)_2O$, K_2CO_3; (j) NBS, DCM; (k) TFA, DCM, then $NaHCO_{3(eq)}$.

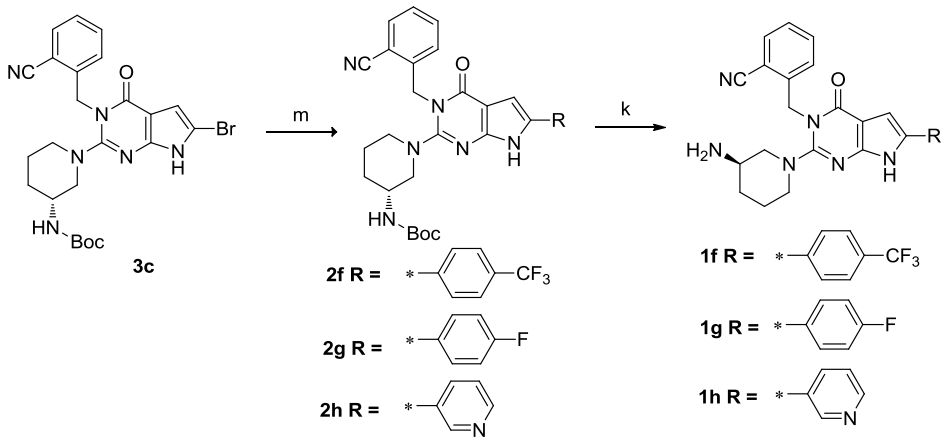
The synthesis of compounds **1d** and **1e** are outlined in Scheme **3**. The synthesis of **1d** and **1e** began with the compound **5a**, which was treated with CH_3I to get the key intermediate **2d**. The following reactions are similar to **1a** and **1b**.

Scheme 3. Synthesis of compounds **1d** and **1e.**



Reagents: (l) NaH, MeI, THF.

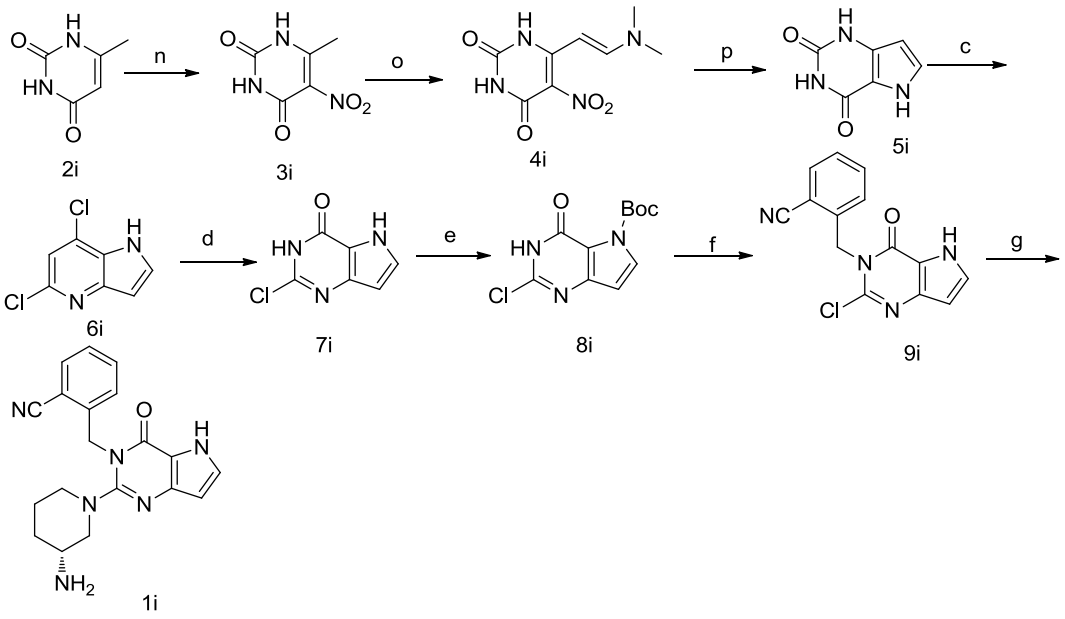
Scheme 4. Synthesis of compounds **1f** – **1h.**



Reagents: (m) Boronic acid, Pd(PPh3)4, t-butylamine, iso-propanol/H_2O.

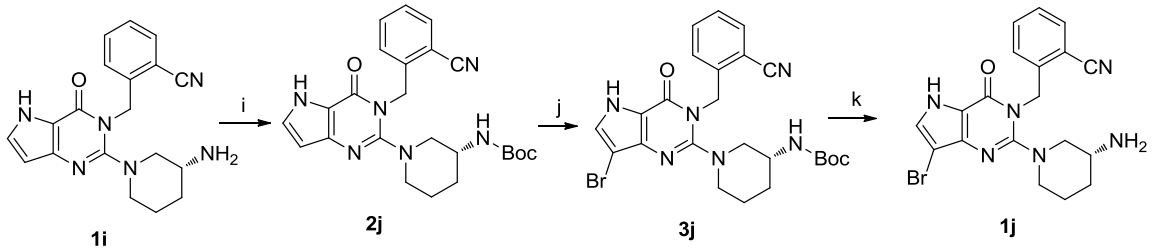
The synthesis of compounds **1i** is outlined in Scheme **4**. The key intermediate **6i** was prepared from 6-methylpyrimidine-2,4(1H,3H)-dione **2i** according to the previously reported procedure[23 专利]. The following reactions are similar to **1a** and **1b**.

Scheme 5. Synthesis of compound **1i.**



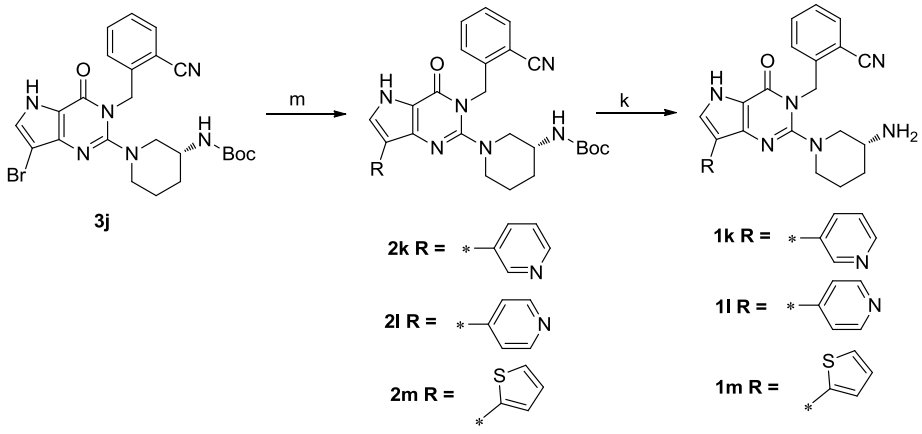
Reagents: (n) sulfuric acid, fuming nitric acid; (o) Dimethylfomamide-dimethyl acetal, DMF; (p) AcOH, Zn.

Scheme 6. Synthesis of compound **1j.**



Reagents: (i) $(Boc)_2O$, K_2CO_3; (j) NBS, DCM; (k) TFA, DCM, then $NaHCO_{3(eq)}$.

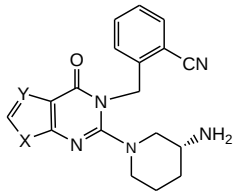
Scheme 7. Synthesis of compounds **1k – 1m.**



Results and discussion

Key scaffold modification of compound 6. In the followed pharmacological evaluations on highly potent compound **6** (IC50=0.33 nM) recently disclosed by our lab, we found it displayed similar anti-diabetic effect with Alogliptin in an oral glucose tolerance test (OGTT) [12]. We assumed the reason to cause such a disconnection between the in vitro activity and the in vivo efficacy probably laid on its poor pharmacokinetic (PK) properties. And the 23.3% oral bioavailability in Sprague Dawley Rats [12] agreed with this assumption. In order to determine and better understand the PK characteristics of this scaffold, we looked into possible causes for low oral bioavailability.

Table 1. 30 min %Remaining in RLM of **6-10**.



No.	X	Y	30 min %Remaining in RLM
6	CH	S	6.6
7	CCH3	S	0
8	S	CH	24.57
9	S	CCH3	3.27
10	S	CCF3	4.6

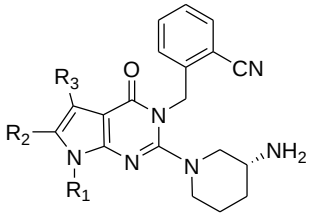
Data are represented as the mean of at least three independent determinations.

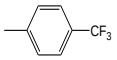
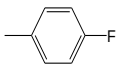
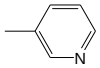
As oral DPP-IV inhibitors, compounds are very likely to suffer from the so called “first pass effect” in the liver. We tested the in vitro metabolic stability of compound **6** using pooled rat liver microsomes (RLM). It displayed a 93% turnover after 30 min incubation, which agreed with our hypothesis of hepatic first pass (Table 1). We initially reasoned the unsubstituted thienyl ring might be one potential site for metabolism and synthesized methyl-substituted compound **7**. Metabolite study showed the methyl substitution helped stabilized the thienyl ring slightly. Yet alkyl substitution didn’t stabilize compounds themselves obviously, nor did electro-withdrawing substitution or structure rotation

(compounds **7-10** in Table 1). Thus we realized the necessary to replace the thienyl ring with a more metabolically robust moiety such as pyrryl ring.

Initially we synthesized a bald pyrrolopyrimidine analogue, compound **1a**, to test the feasibility of this scaffold modification. Gratifyingly it was very stable in the RLM with 65.07% left after 30 min incubation (Table 2). However, compound **1a** had an even worse oral bioavailability (5.7%) in rat than compound **6**. We reckoned on the sharp decrease in bioavailability and realized that although pyrrolopyrimidine scaffold could get rid of hepatic first pass effect. In the same time, it might induce other effect to damage its PK process. The cascade of events determining oral bioavailability is well known today. Substantial evidence reveals that human intestine plays as a big barrier for many oral drugs by means of biotransformation and active secretion by transporters like P-glycoprotein (P-gp) [14]. Thus, the incomplete oral bioavailability of compound **1a** might be the result of poor absorption rather than severe first pass effect. We employed the monolayer of Caco-2 cells as the in vitro model for its being derived from human intestine to evaluate the absorption of compound **1a** (Table 2). Not surprisingly, compound **1a** had a very low permeability from the apical side (A) to the basolateral side (B) and a high transporter mediated efflux from B to A, resulting to an extremely high efflux ratio.

Table 2. 30 min %Remaining in RLM and Caco-2 permeability data of **1a-1h**.



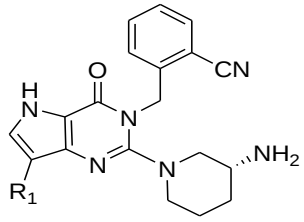
No.	R_1	R_2	R_3	30 min %Remaining in RLM	Caco-2 A-B, $P_{app}\times10^{-6}$ cm/s	Caco-2 B-A, $P_{app}\times10^{-6}$ cm/s	Efflux ratio (B-A/A-B)
1a	H	H	H	65.07	0.64±0.50	12.22±0.12	19.00±0.76
1b	H	CH3	H	91.40	4.36±1.47	9.36±1.90	2.15±0.13
1c	H	Br	H	71.91	5.48±3.90	11.38±5.43	2.08±0.23
1d	CH3	H	H	34.24	1.84±0.54	24.56±3.75	13.32±0.14
1e	CH3	Br	Br	40.95	2.50±0.46	8.41±1.94	3.37±0.05
1f	H		H	92.26	1.73±1.61	23.73±7.21	13.72±0.63
1g	H		H	77.92	0.59±0.54	26.61±2.05	45.27±2.05
1h	H		H	73.75	2.55±1.73	6.01±1.42	2.35±0.44

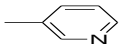
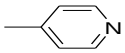
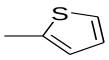
Data are represented as the mean or mean ± SE of at least three independent determinations

Absorptive property optimization of compound 1a. So far there are no direct structure-function relationships or pharmacophore models to describe the structural features responsible for transporter substrates. And the process leading to drug absorption and oral bioavailability is very complex [15]. In our continued way to develop oral bioavailable DPP-IV inhibitors, we adopted several simple strategies based on rules introduced by literatures in hope to improve the absorption and reduce transporter-mediated efflux, which led to a series of pyrrolopyrimidine analogues [16]. Initially we designed compounds **1b** and **1c** by employing alkyl and electro-withdrawing substituents with the hope to create some steric hindrance to reduce the interaction with the transporters. At the same time, incorporation of alkyl groups could increase the lipophilicity to improve the permeability. Data were summarized in Table 2. Compounds **1b** and **1c** showed an improved A-B permeability and significantly lower efflux ratios. At the meantime, different substituents displayed varied B-A efflux ability. Electro-withdrawing groups increased the B-A transport (compound **1a** v.s. **1c** and **1e**). In our further effort to block potential P-gp binding with secondary amine function, we added methyl to 1-position to remove the possible hydrogen bond donor, which led to compounds **1d** and **1e**. Decreased donor would also reduce the desolvation energy cost to increase penetration. Still, 1-position substitution improved the permeability

and decreased the efflux ratio in different degrees. Based on the preliminary data and compound **1c**, which had a lowest efflux ratio of 2.05, we continued to employ some other R_2 groups (compounds **1f-1h**) and evaluated their absorption, among which compound **1h** had an acceptable ratio.

Table 3. 30 min %Remaining in RLM and Caco-2 permeability data of **1i-1m**.



No.	R1	30 min %remaining in RLM	Caco-2 A-B, $P_{app} \times 10^{-6}$ cm/s	Caco-2 B-A, $P_{app} \times 10^{-6}$ cm/s	Efflux ratio (B-A/A-B)
1i	H	39.16	3.44±0.99	14.44±0.81	4.20±0.23
1j	Br	78.05	2.81±1.65	18.41±1.80	6.55±0.49
1k		60.80	0.30±0.09	11.26±1.40	37.78±0.19
1l		28.15	4.13±1.28	10.34±1.82	2.51±0.13
1m		49.90	1.86±0.55	21.20±3.90	11.41±0.11

Data are represented as the mean or mean ± SE of at least three independent determinations.

As the process between compounds and transporters is a configuration interaction. To reduce the possible interaction with P-gp, we synthesized and evaluated the rotated analogues (compounds **1i-m** in Table 3). Generally, the location of hetero atom played a significant role in the interaction. Compound **1i** had a much lower efflux ratio than compound **1a,** which is similar with the situation of compound **6** and **8** (data not showed here). According to the results of compound **1a-h**, we chose several flanking substituents to modify compound **1i** and led to compounds **1j-m** (Table 3).

In vitro pharmacokinetic and biological evaluations. At the same time, we also evaluated the metabolic stability of all pyrrolopyrimidine analogues (Table2 and Table 3). This new scaffold had successfully solved the hepatic first pass effect existed in thienopyrimidine analogues. Yet N-substituted

compounds (**1d-e**) and thienyl, para-pyridyl substituted compounds (**1l-m**) were not quite stable. The latter two groups may cause hepatic metabolism to a certain extent.

Table 4. Inhibitory properties of selected analogues.

No.	DPP-IV IC50 (nM)	DPP-8 IC50 (nM)	DPP-9 IC50 (nM)	hERG (μM)
1b	13.78	>25,000	>25,000	>300
1c	2.64	>25,000	>25,000	>300
1h	1.18	>10,000	>10,000	>300
1j	1.40	>10,000	>10,000	>300
Alogliptin	3.4	>25,000	>25,000	>30[a]

[a]Reported data, see reference 9. IC_{50} values are represented as the mean of at least three independent determinations.

We selected analogues with acceptable in vitro PK properties (efflux ratio less than 7; %remaining more than 60%) to have further biological screening with Alogliptin as a reference compound [11a, 17]. As reported, the selectivity of DPP-IV against DPP-8 and DPP-9 is very critical because the inhibition of these two enzymes may be associated with profound toxicities [18]. Fortunately, all the compounds exhibited more than 1000-fold DPP-IV selectivity versus DPP-8 and DPP-9. In addition, according to the new FDA guidelines for the cardiovascular risk of new therapies for T2D [19], we conducted the human Ether-à-go-go Related Gene (hERG) study. The results were outlined in Table 4. Under our experimental condition, IC_{50} value for Alogliptin was 3.4 nM, which is in accordance with the literature value (IC50<10 nM) [10]. By replacing thienopyrimidine scaffold with pyrrolopyrimidine, we observed a slightly decrease in DPP-IV activity from subnanomolar to nanomolar. Yet all of the pyrrolopyrimidine analogues still exhibited comparable DPP-IV inhibitory activity with IC50 of single-digit nanomolar except compound **1b** (IC50=13.78 nM).

In vivo pharmacokinetic and pharmacodynamic evaluations. We conducted in vivo PK evaluation on compounds **1c**, **h**, and **1j** for their good properties in potency, selectivity and little affinity to hERG channel. Unfortunately the blood plasma concentrations of compound **1h** were too low to detect by LC-

MS/MS. In vivo PK experiments and plasma DPP-IV inhibitory ability were assessed and the results are summarized in Table 5 and Figure 2. Gratifyingly, compound **1c** and **1j** had good PK parameters in rats. Especially compound **1j** inhibited plasma DPP-IV activity more than 50% within 10 h, and had about 30% inhibition of DPP-IV activity lasted for 24 h.

Table 5. Selected PK parameters for compounds **Xc** and **Yb** (hydrochloride) in male Sprague Dawley Rats.

No.	dose(mg/kg) iv/oral	iv $T_{1/2}$ (h)	oral $T_{1/2}$ (h)	poAUC_{0-t} (μg•h•mL^{-1})	CLp ($L•h^{-1}•kg^{-1}$)	$V_{Z/F}$ (L•kg-1)	F%
1c	5/20	2.17 ± 0.36	3.48 ± 0.60	2.50 ± 0.61	7.04 ± 1.60	35.90±12.65	66.3
1j	5/25	1.03 ± 0.30	1.25 ± 0.25	3.55 ± 1.06	7.44 ± 2.59	13.37±5.08	41.02

i.v., intravenous injection; p.o., oral administration.

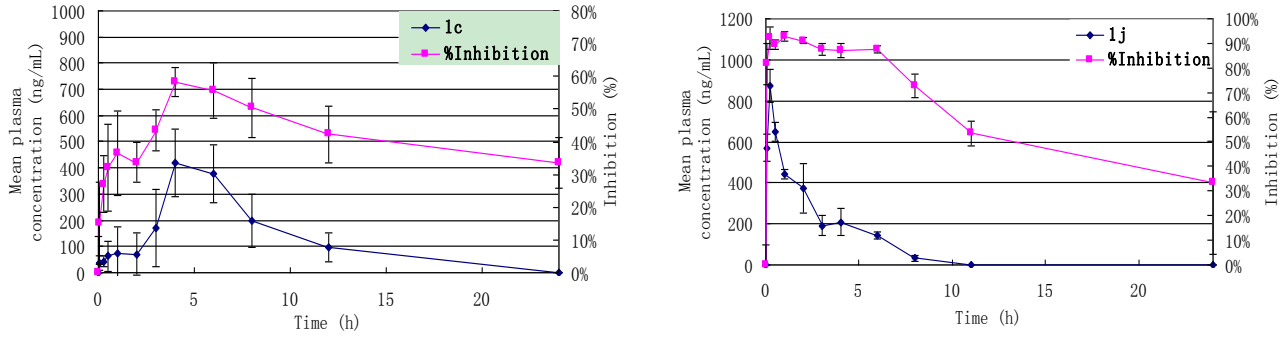


Figure 2. Plasma concentrations and DPP-IV inhibition in rats for compounds **1c** and **1j** (hydrochloride, 20 mg/kg of **1c** and 25 mg/kg of **1j**, p.o.).

Preliminary oral glucose tolerance tests (OGTT) on in ICR and KM mice demonstrated compound **1j** had a better efficacy than compound **1c** and Alogliptin at 1 and 3 mg/kg due to its lower IC_{50} values and stronger plasma DPP-IV inhibition. We deeply evaluated in vivo efficacy of **1j** utilizing ob/ob model mice. Compound **1j** was oral administrated at indicated dose levels 60 min before glucose administration (2 g/kg), and the blood samples drawn and analyzed for the blood glucose concentration at different time intervals from 0-120 min. The glucose AUC was calculated from 0-120 min and OGTT data were summarized in Figure 3. The results demonstrated that **1j** reduced the blood glucose excursion in a dose-dependent manner from 1 mg/kg (), 3 mg/kg () to 9 mg/kg ().

Through a PK-driven optimization on our previously disclosed highly potent DPP-IV inhibitors [12], we have synthesized a new series of potent and selective DPP-IV inhibitors bearing pyrrolopyrimidine scaffold and carried out systematically pharmacological evaluation. We found compound **1j** as a promising candidate for anti-diabetic agents for the further scale-up.

Conclusions

In summary, further pharmacological evaluations on our recently reported DPP-IV inhibitors [12] revealed a disconnection between in vitro activity and in vivo efficacy. In the following optimization, compound **1a** had successfully reduced the hepatic first pass effect by replacing thienyl with pyrryl ring, with a 30 minutes turnover decreased from 93% to 35%. Yet it was found to have very poor intestine absorptive property (efflux ratio= 19 in the monolayer of Caco-2 cells). Further efforts were made to increase the permeability and reduce the transporter mediated efflux, and led to compounds **1b-m**. Among them, compounds **1c, 1h,** and **1j** were found to have good in vitro properties in inhibitory activities, selectivity, and pharmacokinetics. Further PK and PD evaluation came up with the compound **1j**, which had an acceptable PK profile in rats and excellent in vivo efficacy in T2D model mice. This paper reported a successive PK-driven optimization which successfully led to several analogues with drug-like properties represented by compounds **1j**. Further preclinical studies will be carried out on and new analogues with better activity and efficacy may be developed in the future.

Experimental Section

^{1}H NMR spectra were recorded on a Bruker Avance 400. Chemical shifts are expressed in parts per million (ppm), and coupling constants are expressed in Hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br (broad). Low-resolution mass spectra (MS) and compound purity data were acquired on a Waters ZQ LC/MS single quadrupole system equipped with an electrospray ionization (ESI) source, a UV detector (220 nm and 254 nm), and an evaporative light scattering detector (ELSD). Preparative HPLC was conducted on the same system using mixtures of TFA (0.05%) buffered water and acetonitrile. Thin-layer chromatography was performed on 0.25 mm Merck silica gel plates (60F-254) and visualized with

UV light, 5% ethanolic phosphomolybdic acid, ninhydrin or *p*-anisaldehyde solution. Flash column chromatography was performed on silica gel (230-400 mesh, Merck).

7H-pyrrolo[2,3-d]pyrimidine-2,4-diol (4a). Urea (30 g) was added to a solution of sodium ethoxide prepared from sodium (22 g) and absolute ethanol (300 mL) and previously cooled to 5-10°C. The mixture was stirred for 5 min, then treated with ethyl 2-cyano-4,4-diethoxybutanoate 1a (110 g) and allowed to warm with stirring. The sodium salt dissolved, with evolution of heat, and the temperature was kept at 80°C over night. The reaction mixture was cooled and poured into 300 mL of water, and then the solution was concentrated in vacuo. The residue was then acidified with conc. HCl to give a white precipitate, which was collected by filtration, washed with water and dried on a funnel to provide the title compound 4a (66.8 g, yield 92.1%).^{1}H-NMR (400MHz, DMSO-d_6)：δ11.44（1H，s）,11.09 (1H, s), 10.47 (1H, s), 6.56 (1H, t, J=2.4Hz), 6.22 (1H, t, J=2.4Hz)； MS: 152.1$[M+H]^+$.

2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine (5a). The title compound was prepared according to the previously reported procedure (WO2007/012953). A total of 51.20 g of 5a was obtained in 81.3% yield. ^{1}H-NMR (400MHz, DMSO-d_6)：δ12.77（1H，s）, 7.72 (1H, t, J=2.8Hz), 6.65 (1H, dd, J=2.0Hz, 1.6Hz)； MS: 190.0 $[M+H]^+$.

2-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-ol (6a). A mixture of 51.3 g of 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine 5a and 1.36 L 1 N KOH was stirred at 80℃ over night. The solution was then chilled and adjusted to pH 6 with AcOH. The resulting precipitate was collected, washed with water and dried to afford 6a as a solid(37.3 g, yield 80.7%).^{1}H-NMR (400MHz, DMSO-d_6)：δ12.75（1H，br,s）, 12.02 (1H, s), 7.06 (1H, t, J=2.8Hz), 6.45 (1H, t, J=2.8Hz)； MS: 168.0 $[M+H]^+$.

Tert-butyl 2-chloro-4-hydroxy-7H-pyrrolo[2,3-d]pyrimidine-7-carboxylate (7a). 20 g of 2-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-ol **6a** was dissolved in dry THF(400 mL), then the solution was treated with dry Et_3N(16.4 mL) and DMAP(1 g). The mixture was stirred for 30 min at 0 °C, then a solution of $(Boc)_2O$ (27 g) in dry THF (100 mL) was slowly added. The solution was stirred at rt over night, then removed in vacuo and the solution was extracted with ethyl acetate, the organic layer was dried (Na_2SO_4)

and removal of the solvent under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH = 20/1) to give the title compound **7a** (30 g, yield 94.3%). ^{1}H-NMR (400MHz, $CDCl_3$)：δ12.76 (1H, br, s), 7.37 (1H, d, J=4.0Hz), 6.73 (1H, d, J=3.6Hz），1.68（9H，t, J=7.6Hz）；MS: 292.0 $[M+Na]^+$.

2-((2-chloro-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (8a). NaH (2.1 g, 51.6 mmol) was added to a stirred solution of **7a** (8.4 g, 44.9 mmol) in DME (120 mL) and DMF (30 mL) at 0°C. Twenty minutes later, LiBr (7.9 g, 89.7 mmol) was added, and the mixture was allowed to warm to room temperature. After 15 minutes, α-bromo-*o*-tolunitrile (10.15 g, 51.6 mmol) was then added, and the mixture was heated at 65°C overnight. After cooling, the mixture was poured into water (1,000 mL), and then extracted with ethyl acetate, and the organic layer was dried (Na_2SO_4) and removal of the solvent under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH = 20/1) to give the title compound **8a**. ^{1}H-NMR (400MHz, DMSO-d_6)： δ12.20 (1H，s), 7.90 (1H, d，J=7.6Hz)，7.65 (1H, t), 7.50 (1H, t), 7.15 (2H, m), 6.54 (1H，t), 5.58 (2H, s)；MS: 285.0 $[M+H]^+$， 307.0$[M+Na]^+$.

(R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl) benzonitrile (1a). A mixture of **8a** (13.1 g, 43.4 mmol), 3-(*R*)-aminopiperidine dihydrochloride (11.5 g, 66.0 mmol) and $NaHCO_3$ (17.4 g, 173.6 mmol) in 300 mL of ethanol in a sealed tube was heated at 150°C for 6 hours. The reaction mixture was then cooled to room temperature and filtered. The resulting filtrate was concentrated in vacuo and then purified by flash chromatography to give the title compound 1a (10.2 g, yield 63%). ^{1}H-NMR (400MHz, $CDCl_3$)：δ 10.90 (1H, br, s), 7.59 (1H, d, J=7.6Hz), 7.36 (1H, t, J=7.6Hz), 7.25 (1H, t, J=7.6Hz), 6.97 (1H, d, J=7.6Hz), 6.76 (1H, d, J=7.6Hz), 6.59 (1H, d, J=7.6Hz), 5.53 (2H, d, J=5.2Hz), 3.13 (1H, t, J=2.4Hz), 2.96 (2H, m), 2.71 (2H, t), 1.86 (2H, m), 1.69 (1H, m), 1.56 (1H, m);MS: 349.1$[M+H]^+$, 371.1$[M+Na]^+$.

6-methyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diol (4b). A solution of 6-aminopyrimidine-2,4(1H,3H)-dione **3b** (5.0 g) was treated at room temperature with sodium acetate (5.0 g) dissolved in H_2O (200 mL).

The mixture was stirred for 30 min at rt, and the 1-chloro-2-Propanone (5 mL) was added, then the solution was heated to boiling for 72h. The reaction was cooled and filtrated to give the title compound **4b** (5.2 g, yield 80%). MS: 166.0$[M+H]^+$.

4-dichloro-6-methyl-7H-pyrrolo[2,3-d]pyrimidine (5b). In a manner identical to that described for **5a**, the title compound **5b** (400 mg, yield 20%) was prepared. MS: 202.0$[M+H]^+$.

2-chloro-6-methyl-4,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (6b). In a manner identical to that described for **6a**, the title compound **6b** (260 mg, yield 71.6%) was prepared. MS: 184.0$[M+H]^+$.

Tert-butyl 2-chloro-6-methyl-4-oxo-4H-pyrrolo[2,3-d]pyrimidine-7(4aH)-carboxylate (7b). In a manner identical to that described for **7a**, the title compound 7b was prepared.

2-((2-chloro-6-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (8b). In a manner identical to that described for 8a, the title compound 8b was prepared. MS: 299.1$[M+H]^+$.

(R)-2-((2-(3-aminopiperidin-1-yl)-6-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (1b). In a manner identical to that described for 1a, the title compound 1b was prepared. ^{1}H-NMR (400MHz, $CDCl_3$)：δ7.63 (1H, m), 7.42 (1H, m), 7.29 (1H, m), 7.01(1H, m), 6.28 (1H, s), 5.55 (2H, s), 3.14 (1H, m), 2.97 (2H, m), 2.72 (2H, m), 2.30 (3H, s), 1.90 (1H, m), 1.70 (1H, m), 1.58 (1H, m), 1.34 (1H, m); MS: 363.2$[M+H]^+$.

(R)-tert-butyl-2-(3-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3-(2-cyanobenzyl)-4-oxo-3H-pyrrolo [2,3-d]pyrimidine-7(4H)-carboxylate (2c). 348.4 mg of **1a** was dissolved in DCM (20 mL), then the solution was treated with K_2CO_3 (346 mg). The mixture was stirred for 30 min at 0 °C, then a solution of $(Boc)_2O$ (873 mg) in dry DCM (10 mL) was slowly added. The solution was stirred at rt over night, then filtrated. The residue was purified by silica gel chromatography (PE/EA = 2/1) to give the title compound **2c**. MS: 549.3$[M+H]^+$, 571.3$[M+Na]^+$.

(R)-2-((2-(3-aminopiperidin-1-yl)-6-bromo-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (2c) and (R)-2-((2-(3-aminopiperidin-1-yl)-5,6-dibromo-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (1c). 100 mg of 2c was dissolved in DCM (20

mL), and then the solution was treated with NBS (31 mg). The mixture was stirred for 4 h at rt, then removal of the solvent under reduced pressure gave a crude residue, which was purified by silica gel chromatography (DCM) to give the product (R)-tert-butyl(1-(6-bromo-3-(2-cyanobenzyl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-2-yl)piperidin-3-yl)carbamate (**3c**). To a solution of **3c** in DCM was added TFA at 0 °C. After being stirred at room temperature for 3 h, the solution was treated with saturated $NaHCO_3$ to pH 6-7, and then removal of the solvent under reduced pressure gave a crude residue, which was purified by silica gel chromatography (DCM/MeOH) to give the title compound **1c**. ^{1}H-NMR (400MHz, $CDCl_3$)：δ7.64(1H, dd, J=7.6 Hz), 7.36 (1H, t, J=7.6 Hz), 7.30 (1H, t, 7.6 Hz), 6.96 (1H, d, 7.2Hz), 6.58 (1H, s), 5.53 (2H, m), 3.19 (1H, m), 3.06 (1H, m), 2.97 (1H, m), 2.80 (1H, mt), 1.93 (2H, m), 1.76 (2H, m), 1.64(2H, m); MS: 426.9$[M+H]^+$.

2,4-dichloro-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (2d). Into a solution of 60% NaH (750 mg) in dry THF (20mL), a solution of **5a** (1.7 g) in dry THF (50mL) was slowly added and the solution was stirred at 0℃ for 30 min, then MeI (0.79 mL) was added at 0℃. The mixture was stirred at rt over night. 20 mL of water was added and the solution was extracted with ether (3×10 mL) and washed with saturated brine. The organic phase was dried with anhydrous sodium sulphate and filtered. Ether was evaporated in vacuo to gave the a crude residue, which was purified by silica gel chromatography (PE/EA＝10/1) to give the product **2d**(1.53 g, 84.1%). MS: 202.1$[M+H]^+$.

2-chloro-7-methyl-4a,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (3d). In a manner identical to that described for **6a**, the title compound **3d** was prepared. MS: 184.0$[M+H]^+$.

2-((2-chloro-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (4d). In a manner identical to that described for **8a**, the title compound **4d** was prepared. MS: 299.0$[M+H]^+$.

(R)-2-((2-(3-aminopiperidin-1-yl)-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (1d). In a manner identical to that described for **1a**, the title compound **1d** was prepared. ^{1}H-NMR (400MHz, $CDCl_3$)：δ7.64(1H, d, J=7.6 Hz), 7.45 (1H, d, J=7.6 Hz), 7.31 (1H, t, 7.6

Hz), 7.00 (1H, d, 7.6Hz), 6.73 (1H, d, J=3.2 Hz), 6.60 (1H, d, J=3.2 Hz), 5.56 (2H, s), 3.71 (3H, s), 3.15 (1H, m), 3.01 (1H, m), 2.92 (1H, m), 2.74 (1H, m), 2.58 (1H, m), 1.92(1H, m) , 1.72(1H, m) , 1.64 (1H, m) , 1.44(2H, s) , 1.20(1H, m); MS: 363.2$[M+H]^+$.

(R)-tert-butyl(1-(3-(2-cyanobenzyl)-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-2-yl)piperidin-3-yl)carbamate (2e). In a manner identical to that described for **2c**, the title compound **2e** was prepared.

(R)-2-((2-(3-aminopiperidin-1-yl)-5, 6-dibromo-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d] pyrimidin-3-yl)methyl)benzonitrile (1e). In a manner identical to that described for **1c**, the title compound **1e** was prepared. ^{1}H-NMR (400MHz, $CDCl_3$)：δ7.65(1H, dd, J=7.6 Hz), 7.46 (1H, t, J=7.6 Hz), 7.32 (1H, t, 7.6 Hz), 7.04 (1H, d, 7.6Hz), 6.66 (1H, s), 5.51 (2H, s), 3.80 (1H, s), 3.68 (3H, m), 3.29 (1H, m), 3.17 (1H, s), 3.01 (3H, m), 1.78(2H, m) , 1.65 (2H, m) , 1.58(1H, m); MS: 441.0$[M+H]^+$.

Coupling Reaction Affording Compounds 2f-2h and 2k-2m; General Procedure. (R)-tert-butyl (1-(6-bromo-3-(2-cyanobenzyl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-2-yl)piperidin-3-yl) carbamate (**3c**) or (R)-2-((2-(3-aminopiperidin-1-yl)-7-bromo-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (**3j**) (500 mg, 0.95 mmol) was mixed with arylboronic acid (1.42 mmol), $Pd(PPh_3)_4$ (77 mg, 0.0665 mmol) and t-butylamine (4 mL) in iso-propanol-H_2O (2:1), flushed with argon for 5 min, and the mixture was then stirred and refluxed (oil bath, 100 °C) in sealed tube until the starting material had disappeared (TLC monitoring). After cooling, the solution was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel.

General Procedure for tert-Butyloxycarbonyl (Boc) Deprotection. To a solution of **2f-2h** or **2k-2m** in DCM, TFA was slowly added and the solution was stirred at 0 °C. After being stirred at room temperature for 2 h, saturated $NaHCO_3$ was added to make pH 7 by stirring and in ice bath. The solution was extracted with ethyl acetate, the organic layer was dried (Na_2SO_4) and removal of the solvent under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH = 15/1) to give the compounds **1f-1h** or **1k-1m**.

1f:

1g:

1h: ^{1}H-NMR (400MHz, MeOD)：δ 9.00(1H, m), 8.69(1H, m), 8.22 (1H, m), 8.10(1H, m), 7.72 (1H, d, J = 7.6 Hz), 7.61 (1H, t, J = 7.2 Hz), 7.43 (1H, t, J = 7.2 Hz), 7.28 (2H, m), 5.59 (1H, d, J = 15.2 Hz), 5.50 (1H, d, J = 15.2 Hz), 3.68 (1H, m), 3.54 (1H, m), 3.22 (2H, m), 3.00 (1H, m), 2.16 (1H, m), 1.83(1H, m), 1.67 (2H, m); MS: 426.1$[M+H]^+$.

1k: 1H-NMR (400MHz, DMSO-d_6): δ 12.66 (1H, s), 9.55 (1H, s), 9.10-9.12 (1H, d, J = 8 Hz), 8.69-8.70 (1H, d, J = 4 Hz), 8.34 (3H, s), 8.27-8.28 (1H, d, J = 4 Hz), 7.99-8.03 (1H, t, J = 8 Hz), 7.83-7.85 (1H, d, J = 8 Hz), 7.59-7.63 (1H, t, J = 8 Hz), 7.43-7.47 (1H, t, J = 8 Hz), 7.11-7.13 (1H, d, J = 8 Hz), 5.42-5.53 (2H, dd, J = 24 Hz, J = 16 Hz), 3.57 (1H, s), 3.60 (2H, s), 3.10-3.13 (1H, d, J = 8 Hz), 2.95 (1H, s), 1.99 (1H, s), 1.85 (1H, s), 1.58-1.60 (1H, d, J = 8 Hz); MS: 426.2, 427.2, 428.2 $[M+1]^+$.

1l:

1m:

5,7-dichloro-1H-pyrrolo[3,2-b]pyridine (6i). The title compound was prepared from 6-methylpyrimidine-2,4(1H,3H)-dione **2i** according to the previously reported procedure (WO2009/062258). MS: 187.9$[M+H]^+$.

2-chloro-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (7i). The title compound was prepared from 5,7-dichloro-1H-pyrrolo[3,2-b]pyridine **6i** in 94% yield by a method analogous to that used to make **6a**. MS: 170.0$[M+H]^+$.

Tert-butyl 2-chloro-4-oxo-3H-pyrrolo[3,2-d]pyrimidine-5(4H)-carboxylate (8i). The title compound was prepared from 2-chloro-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one **7i** in 57% yield by a method analogous to that used to make **7a**. MS: 292.0$[M+Na]^+$.

2-((2-chloro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (9i). The title compound was prepared from tert-butyl 2-chloro-4-oxo-3H-pyrrolo[3,2-d]pyrimidine-5(4H)-carboxylate **8i** in 90% yield by a method analogous to that used to make **8a**. ^{1}H-NMR (400MHz, $CDCl_3$)：δ7.73 (1H, m), 7.53 (1H, m), 7.39 (2H, m), 7.12 (1H, d), 6.53 (1H, m), 5.79 (2H, s).

(R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl) benzonitrile (1i). The title compound was prepared from 2-((2-chloro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d] pyrimidin-3-yl)methyl)benzonitrile **9i** in 75% yield by a method analogous to that used to make **1a**. ^{1}H-NMR (400MHz, $CDCl_3$)：δ7.66 (1H, m), 7.43 (1H, m), 7.31 (1H, m), 7.24 (1H, d), 6.93 (1H, d), 6.41 (1H, d), 5.62 (2H, s), 3.15 (1H,m), 2.94 (2H, m), 2.79 (1H, t), 2.66 (1H, m), 1.91 (1H, m), 1.74 (1H, m), 1.59 (1H, m), 1.23 (1H, m); MS: 349.1$[M+H]^+$.

(R)-2-((2-(3-aminopiperidin-1-yl)-7-bromo-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (1j). The title compound was prepared from (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile **1i** by a method analogous to that used to make **1c**. ^{1}H-NMR (400MHz, MeOD+DMSO-d_6) δ: 8.04-8.06 (1H, d, J = 8 Hz) , 7.86-7.90 (1H, t, J = 8 Hz), 7.70-7.74 (2H, m), 7.40-7.42 (1H, d, J = 8 Hz) , 5.73-5.91 (2H, dd, J = 15 Hz, J = 15 Hz), 3.75-3.78 (1H, m), 3.65-3.71 (1H, m), 3.38-3.46 (2H, m), 3.15-3.20 (1H, m), 2.35-2.37 (1H, m), 2.11-2.15 (1H, m), 1.92-1.98(2H, m); MS: 427.0, 428.0, 429.0$[M+1]^+$.

Caco-2 permeability bidirectional study. Test compounds (10 μM) were added to either the apical or basolateral side of a confluent monolayer of Caco-2 cells. Permeability was measured by monitoring the appearance of the compounds by LC-MS/MS on the other side of the cell membrane after 60 min incubation.

Inhibition of DPP-IV, DPP-8 and DPP-9 in vitro. Solutions of test compounds in varying concentrations（≤10 mM, final concentration) were prepared in Dimethyl Sulfoxide (DMSO) and then diluted into assay buffer comprising: 20mM Tris, pH 7.4; 20 mM KCl; and 0.1mg/mL BSA. Human DPP-IV (0.1 nM, final concentration) was added to the dilutions and pre-incubated for 10 minutes at ambient temperature before the reaction was initiated with Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; Sigma-Aldrich; 10 μM, final concentraton). The total volume of the reaction mixture was 100 μL. the reaction was followed kinetically (excitation at 400 nm; emission at 505 nm) for 5-10

minutes or an end-point was measured after 10 minutes. Inhibition constants (IC_{50}) were calculated from the enzyme progress curves using standard mathematical models

Microsomal stability. Test compounds (1 mM, final concentration) were incubated with rat pooled liver microsomes (3mg/mL, final concentration) for 5 mins. Samples were removed at five time points (0, 2, 4, 12, 20, 30min) and the concentration of parent compounds was determined by LC-MS/MS.

In vivo pharmacokinetic study. Adult male SD rats (n= 4/group) were administrated with the test compounds dissolved in distilled water at a single dose of 20 mg/kg or 25 mg/kg for oral and 5mg/mL in veil. Blood samples of 100-200 μL were collected from the orbit at the time points indicated in Figure 2. The blood concentration of test compounds was determined by LC-MS/MS. The PK parameters were obtained from the pharmacokinetic software DAS. 2.0.

DPP-IV inhibition in SD rats. Adult male SD rats (n=4/group) were orally gavaged with the test compounds dissolved in distilled water at a single dose of 20 mg/kg or 25 mg/kg. Blood samples of 20-25 μL were collected from the orbit at the time points indicated in Figure 2 and the plasma fraction was kept frozen until DPP-IV activity measurement. The plasma DPP-IV activity was determined by cleavage rate of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; Sigma-Aldrich). Plasma (10 μL) was mixed with 140 μL of 150 μM Gly-Pro-AMC in assay buffer that was composed of 25mM tris(hydroxymethyl)-aminomethane HCl (PH 7.4), 140 mM NaCl, 10 mM KCl and 0.1% bovine serum albumin, the fluorescence was determined by using Thermo Scientific Fluoroskan Ascent FL (excitation at 400 nm and emission at 505 nm). DPP-IV inhibition in plasma was described as (vehicle control activity- remained activity)/vehicle control activity. one unit of activity is defined as the amount of enzyme that produces 1 μM products per minute.

OGTT in ob/ob Mice. Male 13-14 week-old ob/ob mice (Jackson Laboratories) were maintained under constant temperature and humidity conditions, a 12 h/12 h light-dark cycle, and had free access to a 10% fat rodent diet (D1245B Research Diets) and tap water. After an overnight fasting period, animals were dosed orally with vehicle (water) or DPP4 inhibitor (1, 3, 9 g/kg) at -60 min. Two blood samples were collected at-60 and 0 min by tail bleed for glucose determinations. Glucose (2 g/kg) was then

administered orally (at 0 min). Additional blood samples were collected at 15, 30, 60, and 120 min for glucose determinations. Plasma glucose was determined with an Accu-Chek Advantage (Roche) glucometer. Data represent the mean of at least 6 mice/group. Data analysis was performed using one way ANOVA followed by Dunnett's test. All procedures were performed according to GIBH-IACUC guidelines.

Cytochrome P450 inhibition assay. Test compounds (0.1-100 μM) is incubated with human pooled liver microsomes and NADPH in the presence of a specific CYP P450 probe substrate. Each reaction is performed under linear conditions with respect to time and microsomal protein concentrations. The metabolites are monitored by LC-MS/MS. A decrease in the formation of the metabolite compared to the vehicle control is used to calculate an IC_{50} using the software Grafit 5.

Acknowledgement. This research was supported by the Bureau of Science and Technology of Guangzhou Municipality, China, by Grant No. 2009Z1-E871.

Supporting Information Available: The contents of Supporting Information may include the following: (1) large tables, (2) extensive figures, (3) lengthy experimental procedures, (4) mathematical derivations, (5) analytical and spectral characterization data, (6) molecular modeling coordinates, (7) modeling programs, (8) crystallographic information files (CIF), (9) instrument and circuit diagrams, (10) and expanded discussions of peripheral findings. For complete instructions on how to prepare this material for publication, check the Guide, Notes, Notice, or Instructions for Authors that appear in each publication's first issue of the year and on the World Wide Web at http://pubs.acs.org.

References

1. Hollander, P. A.; Kushner, P., Type 2 diabetes comorbidities and treatment challenges: rationale for DPP-4 inhibitors. *Postgrad Med* **2010,** *122* (3), 71-80.
2. (a) Knudsen, L. B., Glucagon-like peptide-1: the basis of a new class of treatment for type 2 diabetes. *J Med Chem* **2004,** *47* (17), 4128-34; (b) Drucker, D. J., Minireview: the glucagon-like peptides. *Endocrinology* **2001,** *142* (2), 521-7.

3. (a) Holst, J. J.; Deacon, C. F., Glucagon-like peptide 1 and inhibitors of dipeptidyl peptidase IV in the treatment of type 2 diabetes mellitus. *Curr Opin Pharmacol* **2004,** *4* (6), 589-96; (b) Drucker, D. J., Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology* **2002,** *122* (2), 531-44.
4. (a) Kieffer, T. J.; McIntosh, C. H.; Pederson, R. A., Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* **1995,** *136* (8), 3585-96; (b) Deacon, C. F.; Nauck, M. A.; Toft-Nielsen, M.; Pridal, L.; Willms, B.; Holst, J. J., Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* **1995,** *44* (9), 1126-31.
5. (a) Abel, T.; Feher, J., [A new therapeutic possibility for type 2 diabetes: DPP-4 inhibitors (sitagliptin)]. *Orv Hetil* **2010,** *151* (25), 1012-6; (b) Deacon, C. F.; Holst, J. J.; Carr, R. D., Glucagon-like peptide-1: a basis for new approaches to the management of diabetes. *Drugs Today (Barc)* **1999,** *35* (3), 159-70; (c) Gallwitz, B., [Diabetes therapy--novel drugs to come (DPP-4 inhibitors, GLP-1 agonists, SGLT-2 inhibitors)]. *MMW Fortschr Med* **2010,** *152* (20), 43-4.
6. (a) Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E., (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin -7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* **2005,** *48* (1), 141-51; (b) Kim, D.; Kowalchick, J. E.; Brockunier, L. L.; Parmee, E. R.; Eiermann, G. J.; Fisher, M. H.; He, H.; Leiting, B.; Lyons, K.; Scapin, G.; Patel, S. B.; Petrov, A.; Pryor, K. D.; Roy, R. S.; Wu, J. K.; Zhang, X.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E., Discovery of potent and selective dipeptidyl peptidase IV inhibitors derived from beta-aminoamides bearing subsituted triazolopiperazines. *J Med Chem* **2008,** *51* (3), 589-602.
7. (a) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Hughes, T. E., 1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(S)-pyrrolidinecarbon itrile: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem* **2002,** *45* (12), 2362-5; (b) Bosi, E.; Camisasca, R. P.; Collober, C.; Rochotte, E.; Garber, A. J., Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin. *Diabetes Care* **2007,** *30* (4), 890-5; (c) Fonseca, V.; Schweizer, A.; Albrecht, D.; Baron, M. A.; Chang, I.; Dejager, S., Addition of vildagliptin to insulin improves glycaemic control in type 2 diabetes. *Diabetologia* **2007,** *50* (6), 1148-55.
8. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S. P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G., Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* **2005,** *48* (15), 5025-37.
9. Eckhardt, M.; Langkop, E.; Mark, M.; Tadayyon, M.; Thomas, L.; Nar, H.; Pfrengle, W.; Guth, B.; Lotz, R.; Sieger, P.; Fuchs, H.; Himmelsbach, F., 8-(3-(R)-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione (BI 1356), a highly potent, selective, long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes. *Journal of Medicinal Chemistry* **2007,** *50* (26), 6450-6453.
10. Feng, J.; Zhang, Z.; Wallace, M. B.; Stafford, J. A.; Kaldor, S. W.; Kassel, D. B.; Navre, M.; Shi, L.; Skene, R. J.; Asakawa, T.; Takeuchi, K.; Xu, R.; Webb, D. R.; Gwaltney, S. L., 2nd, Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV. *J Med Chem* **2007,** *50* (10), 2297-300.

11. (a) Nordhoff, S.; Bulat, S.; Cerezo-Galvez, S.; Hill, O.; Hoffmann-Enger, B.; Lopez-Canet, M.; Rosenbaum, C.; Rummey, C.; Thiemann, M.; Matassa, V. G.; Edwards, P. J.; Feurer, A., The design of potent and selective inhibitors of DPP-4: optimization of ADME properties by amide replacements. *Bioorg Med Chem Lett* **2009,** *19* (22), 6340-5; (b) Tsai, T. Y.; Hsu, T.; Chen, C. T.; Cheng, J. H.; Yeh, T. K.; Chen, X.; Huang, C. Y.; Chang, C. N.; Yeh, K. C.; Hsieh, S. H.; Chien, C. H.; Chang, Y. W.; Huang, C. H.; Huang, Y. W.; Huang, C. L.; Wu, S. H.; Wang, M. H.; Lu, C. T.; Chao, Y. S.; Jiaang, W. T., Novel trans-2-aryl-cyclopropylamine analogues as potent and selective dipeptidyl peptidase IV inhibitors. *Bioorg Med Chem* **2009,** *17* (6), 2388-99; (c) Wang, L.; Zhang, B.; Ji, J.; Li, B.; Yan, J.; Zhang, W.; Wu, Y.; Wang, X., Synthesis and evaluation of structurally constrained imidazolidin derivatives as potent dipeptidyl peptidase IV inhibitors. *Eur J Med Chem* **2009,** *44* (8), 3318-22.
12. Deng, J.; Peng, L.; Zhang, G.; Lan, X.; Li, C.; Chen, F.; Zhou, Y.; Lin, Z.; Chen, L.; Dai, R.; Xu, H.; Yang, L.; Zhang, X.; Hu, W., The highly potent and selective dipeptidyl peptidase IV inhibitors bearing a thienopyrimidine scaffold effectively treat type 2 diabetes. *Eur J Med Chem* **2011,** *46* (1), 71-6.
13. Liu, H.; Ko, S. B.; Josien, H.; Curran, D. P., Selective N-Functionalization of 6-Substituted-2-Pyridones. *Tetrahedron Lett* **1995,** *36* (49), 8917-8920.
14. (a) Takano, M.; Yumoto, R.; Murakami, T., Expression and function of efflux drug transporters in the intestine. *Pharmacol Ther* **2006,** *109* (1-2), 137-61; (b) Murakami, T.; Takano, M., Intestinal efflux transporters and drug absorption. *Expert Opin Drug Metab Toxicol* **2008,** *4* (7), 923-39; (c) Ho, R. H.; Kim, R. B., Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharmacol Ther* **2005,** *78* (3), 260-77; (d) Chan, L. M.; Lowes, S.; Hirst, B. H., The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. *Eur J Pharm Sci* **2004,** *21* (1), 25-51.
15. (a) Ekins, S.; Swaan, P. W., Development of computational models for enzymes, transporters, channels, and receptors relevant to ADME/Tox. *Rev Comp Ch* **2004,** *20*, 333-415; (b) Stouch, T. R.; Gudmundsson, O., Progress in understanding the structure-activity relationships of P-glycoprotein. *Adv Drug Deliv Rev* **2002,** *54* (3), 315-28; (c) Varma, M. V.; Perumal, O. P.; Panchagnula, R., Functional role of P-glycoprotein in limiting peroral drug absorption: optimizing drug delivery. *Curr Opin Chem Biol* **2006,** *10* (4), 367-73; (d) Pajeva, I. K.; Globisch, C.; Wiese, M., Structure-function relationships of multidrug resistance P-glycoprotein. *J Med Chem* **2004,** *47* (10), 2523-33.
16. (a) Winiwarter, S.; Bonham, N. M.; Ax, F.; Hallberg, A.; Lennernas, H.; Karlen, A., Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. *J Med Chem* **1998,** *41* (25), 4939-49; (b) Palm, K.; Stenberg, P.; Luthman, K.; Artursson, P., Polar molecular surface properties predict the intestinal absorption of drugs in humans. *Pharm Res* **1997,** *14* (5), 568-71; (c) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **2001,** *46* (1-3), 3-26; (d) vandeWaterbeemd, H.; Camenisch, G.; Folkers, G.; Raevsky, O. A., Estimation of Caco-2 cell permeability using calculated molecular descriptors. *Quant Struct-Act Rel* **1996,** *15* (6), 480-490.
17. Nordhoff, S.; Lopez-Canet, M.; Hoffmann-Enger, B.; Bulat, S.; Cerezo-Galvez, S.; Hill, O.; Rosenbaum, C.; Rummey, C.; Thiemann, M.; Matassa, V. G.; Edwards, P. J.; Feurer, A., From lead to preclinical candidate: optimization of beta-homophenylalanine based inhibitors of dipeptidyl peptidase IV. *Bioorg Med Chem Lett* **2009,** *19* (16), 4818-23.
18. Cho, T. P.; Long, Y. F.; Gang, L. Z.; Yang, W.; Jun, L. H.; Yuan, S. G.; Hong, F. J.; Lin, W.; Liang, G. D.; Lei, Z.; Jing, L. J.; Shen, G. A.; Hong, S. G.; Dan, W.; Ying, F.; Ke, Y. P.; Ying, L.; Jun, F.; Tai, M. X., Synthesis and biological evaluation of azobicyclo[3.3.0] octane derivatives as dipeptidyl peptidase 4 inhibitors for the treatment of type 2 diabetes. *Bioorg Med Chem Lett* **2010,** *20* (12), 3565-8.
19. (a) Raschi, E.; Vasina, V.; Poluzzi, E.; De Ponti, F., The hERG K+ channel: target and antitarget strategies in drug development. *Pharmacol Res* **2008,** *57* (3), 181-95; (b) Pearlstein, R.; Vaz, R.; Rampe, D., Understanding the structure-activity relationship of the human ether-a-go-go-related gene cardiac K+ channel. A model for bad behavior. *J Med Chem* **2003,** *46* (11), 2017-22.